

# ANTIMICROBIAL FACTORS IN TISSUES AND PHAGOCYTTIC CELLS

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## INTRODUCTION

Even in this modern sanitary age, man lives in an environment literally swarming with microbes. Skin and mucous membranes, being in contact with the outside world, are heavily infested with bacteria. Until fairly recently, normal skin and mucosal surfaces were thought to pose an impenetrable barrier to passage of microorganisms. We know now, however, that bacteria in small numbers frequently cross these "intact" anatomical coverings. Exemplifying this situation is the repeated penetration of small numbers of coliform organisms through the gut wall to reach regional lymph nodes and rarely even the blood stream in normal animals (1-3), and the transient bacteremia with normal oral flora which frequently occurs in man following such everyday activity as chewing or brushing the teeth (4-6). Thus various tissues of the body are probably exposed many times daily to an assortment of microbes. Why, in the vast majority of instances, do these "invaders" fail to multiply in the tissues and cause disease?

There are two possible reasons for failure of bacteria to thrive once they have been seeded in tissues: (a) the tissue might be lacking in a nutrient essential for microbial growth; or (b) the tissue might contain agents or conditions which inhibit reproduction or even survival of the parasite.

That tissues might be lacking in nutrients required for bacterial growth was suggested early in the history of bacteriology. For instance, Pasteur hypothesized that resistance to anthrax of animals receiving prior immunization with attenuated strains was due to utilization by the attenuated bacteria of an essential growth factor which was not regenerated by the host. This concept was, however, soon disproved by Chauveau's observation that a sufficiently large challenge dose of virulent anthrax would overcome natural or acquired immunity to this microorganism. Relationships between the nutritional status of the host and resistance to infection have recently been extensively reviewed (7). As concluded in this review, some experimental evidence suggests

that host resistance to malaria may be enhanced by a deficiency of *p*-aminobenzoic acid, and that tissues of vitamin-deficient animals may be less suitable than normal ones for supporting growth of certain viruses; otherwise, there is little evidence to support the notion that microbes are eliminated or suppressed in tissues because of an inadequate nutritional supply.

Rather it seems more likely that tissues contain agents which inhibit the parasites. From the classical point of view these tissue antimicrobial factors have been considered to be primarily phagocytes and antibodies. There is, however, abundant evidence that factors other than antibodies and phagocytic cells operate in this regard. For example, consider the phenomenon of organ or tissue localization so common to infectious diseases. Streptococci, pneumococci, and meningococci all invade via the upper respiratory tract, yet typically produce disease in, respectively, the pharynx, the lungs, and the meninges. Almost all pathogenic microbes similarly show predilection for certain sites in the host. Since antibodies and phagocytes are available to all parts of the body via the blood stream, these agencies can hardly be directly related to tissue site selection of parasites. Intimate biochemical conditions in normal tissues must differ in such a manner as to render certain areas particularly suitable for proliferation of a given microbial species.

Observations on inflamed or necrotic tissues also suggest that the chemical microenvironment plays a decisive role in limiting growth of microorganisms. In a caseous tubercle, for instance, bacilli disappear from the avascular acellular central zone, while they continue to multiply at the margins where phagocytes and antibodies are plentiful. Therefore, the central caseous area must be lacking in essential nutrients for tubercle bacilli, or contain antimycobacterial substances, or both factors may be operative.

Quite recently Skarnes and Watson reviewed thoroughly present knowledge of antimicrobial substances of normal tissues and fluids (8). No significant discoveries have been made in this field since their report. The present discussion

will not, therefore, attempt a comprehensive review but rather will consider certain tissue and cell substances potentially toxic for microorganisms, stressing biochemical classification of these agents and speculating on the possibility of their antimicrobial activity *in vivo*.

#### ANTIMICROBIAL SUBSTANCES IN NORMAL TISSUES

Several chemical components of normal tissue have been shown to exert antibacterial action under certain conditions *in vitro*. The length of the list of such substances, as related subsequently, should not delude us into thinking our knowledge in this important area is adequate; as will be pointed out presently, we can make few if any reliable statements about the precise biochemical conditions which determine susceptibility to infection *in vivo*.

The concentration of oxygen undoubtedly has an effect on fate of certain bacteria in tissues. As an obvious example, obligatory anaerobes do not multiply in normal well oxygenated areas, whereas their growth may be rapid and fatal to the host when they are situated in necrotic tissue deprived of its oxygen supply. In similar fashion, microbes which require oxygen may be limited in their growth at certain sites in the body because of a marginal oxygen tension; relative susceptibility of various organs to tuberculosis, and fate of tubercle bacilli in caseous areas are, in the opinion of many investigators, determined at least in part by the availability of oxygen to these obligatorily aerobic bacilli (9, 10).

Several observations suggest that the carbon dioxide content of tissues may also influence the fate of microbes (11). Some bacteria, for example *Brucella* spp., require relatively high concentrations of carbon dioxide for optimal growth; their localization *in vivo* may be determined, to some extent, by availability of this gas. On the other hand, excessive local carbon dioxide tension exerts an inhibitory effect on growth of other microbes, such as the tubercle bacillus (10, 12), leading to the concept that infection with these parasites is more likely to occur at tissue sites where accumulation of this gas is uncommon, *e.g.*, the lung. High concentrations of carbon dioxide in the caseous area may be partly responsible for the limitation of growth of mycobacteria in this situation.

Fatty acids have long been recognized to exert antibacterial effects in the laboratory. These

substances may be divided for purposes of discussion into two groups, long chain and short chain. Justification for this division is based on a probable difference in mechanism of antibacterial action, some short chain members acting as metabolic inhibitors, whereas the long chain ones probably kill primarily by virtue of their surface active properties. Fatty acids are widely distributed in all mammalian tissues, where their concentration varies depending on the particular site and the metabolic state. Many bacteria are suppressed in growth or killed *in vitro* by low concentrations of long chain fatty acids (13). These noxious effects on bacteria may be counteracted by inclusion in the test system of various materials, such as serum albumin, which bind the fatty acids. Insufficient information is available on the concentration of *free* fatty acids, *i.e.*, unesterified and not bound to albumin or other substance, in tissues to permit any conclusions regarding a possible antimicrobial role for these compounds *in vivo*.

Several short chain monocarboxylic organic acids exert an antibacterial action on various gram-positive organisms (14, 15). Certain members of this group, for instance lactic acid, are present in some tissues in concentrations higher than those which suppress bacteria *in vitro*. Their action on susceptible parasites is enhanced at acid pH, and is antagonized by the presence of keto- or polycarboxylic acids (15). Here again it is difficult to estimate the importance of these organic acids in contributing to host resistance to infection, because their antibacterial effects are dependent on aspects of the local biochemical environment in tissues which have not been studied adequately.

Lipids of the sterol type also are capable of exerting bactericidal action. For example, bile salts have long been known to kill and bring about lysis of pneumococci. Unfortunately, little information is available about possible antimicrobial effects of the many other sterolic compounds present in tissues.

Antibacterial activity of a high order under certain conditions *in vitro* is recognized for certain porphyrins related to heme (8, 16). This activity is exhibited by the free porphyrins but not by the naturally occurring porphyrin-protein complexes. It remains a matter for speculation whether or not hemoglobin or the various porphyrin-contain-

ing enzymes in the body may dissociate *in vivo* to yield components with antimicrobial powers.

Various proteins of tissues exert inimical effects on microorganisms under laboratory conditions. The most thoroughly studied and most highly purified of these antimicrobial proteins is lysozyme. This enzyme, which was discovered by Fleming (17), is a low molecular weight basic protein which hydrolyzes certain complex acetylaminopolysaccharides (18). The relatively few bacterial species whose cell wall is composed of susceptible substrate are rapidly dissolved by lysozyme. Furthermore, it has recently been demonstrated that lysozyme, acting in concert with certain other substances present in tissues such as chelating agents (19) or acid (C. H. Lack and J. G. Hirsch, *unpublished data*), can kill or suppress growth of a much wider range of microorganisms. Lysozyme is widely distributed in tissues and acts within the physiological range of pH and ionic concentrations; it seems likely that lysozyme may kill bacteria in at least some situations *in vivo*.

Two groups of antimicrobial basic peptides or low molecular weight proteins have been extracted from tissues. One is especially rich in lysine (20, 21), whereas the other contains a high proportion of arginine (22); they have thus come to be known as the polylysine and polyarginine peptides. Their activity *in vitro* has been studied by various techniques, and there is no doubt that quite low concentrations kill some microbes in a suitable setting. Both these peptides are extracted from tissues with relatively strong acid and there is some question as to their existence as free substances in intact animals. Furthermore, their antibacterial activity is readily antagonized by the presence of certain acid polysaccharides (21, 23) or certain inorganic anions (24), making it more difficult to evaluate possible action *in vivo*.

Polylysine and polyarginine peptides are closely related to and in fact may well be derived from histones. The arginine-rich histone B of Crampton *et al.* (25) manifests impressive bactericidal action on several gram-negative organisms under certain laboratory conditions (26). This effect of histone is dependent upon the ionic concentration and the general composition of the medium; salt concentrations only slightly higher than physiological abolish the bactericidal action. The salt effect, considered with the fact that histone ordinarily exists in tissues firmly bound to nucleic

acids and not free, makes it dubious that histone has a role as an antibacterial agent in normal tissues.

Another widely distributed animal protein with demonstrable bactericidal properties is globin. Under rigidly defined conditions of acid pH and low ionic strength, globins from various species kill enteric bacilli (27). The conditions required for this lethal action on bacteria are so far removed from those existing in host tissues that any antimicrobial function for globin *in vivo* seems highly unlikely.

Protamines have long been known to exert antibacterial effects *in vitro*. Present knowledge seems to limit the distribution of protamines to sperm, so that we need not further discuss here the role of these materials as general tissue antimicrobial agents.

The naturally occurring aliphatic amines, spermine and spermidine, exert noxious effects on bacteria in laboratory test systems (28, 29). This antimicrobial action of spermine and its derivative seems to operate by at least two distinct means. In studies on the tubercle bacillus it has been established that spermine added to the test system is oxidized by a specific amine oxidase; a product of this reaction, rather than the spermine itself, is responsible for the observed suppression of growth of mycobacteria (30). The antibacterial product has not yet been identified with certainty, but recent studies present evidence suggesting that it may be an unstable amine aldehyde (31). On the other hand spermine appears to act directly, without intervention by the oxidase, to inhibit the growth of staphylococci (32). Spermine is present in many tissues in concentrations higher than that required for antimicrobial action *in vitro*; its possible activity *in vivo* remains speculative, however, since spermine in tissues may be firmly bound or its action on microorganisms may be influenced by the presence or absence of other materials in the environment, *e.g.*, spermine oxidase and acid polysaccharides.

Antibacterial activity has also been reported for many crude extracts made from such tissues as platelets (8) and lymph nodes (33). The active ingredients in these various extracts have not been sufficiently purified and studied to determine whether they differ from the tissue antimicrobial substances already discussed.

ANTIMICROBIAL SUBSTANCES IN  
PHAGOCYtic CELLS

Over 50 years ago Metchnikoff wrote: "I have always openly acknowledged that the question as to what substances within the phagocytes harm and destroy the microbes is still quite undecided. They may be ferments, digestive or otherwise, or they may be substances, acid or alkaline, completely different from ferments. We shall have to find new and more perfect methods before being able to solve this delicate problem" (34, p. 208). Numerous investigations carried out in the twentieth century have shed some light on this problem, but our knowledge of intracellular antibacterial action remains incomplete.

The first antimicrobial agent recognized within phagocytes was acid. Metchnikoff concluded that the reaction about engulfed particles in leucocyte cytoplasm was acid on the basis of his studies of phagocytosis of litmus granules. These observations have since been extended by Rous and others (16). Several investigators, utilizing supravital indicator dyes, have obtained values ranging from approximately pH 3 to pH 6 for the reaction in the vicinity of engulfed particles. Many microorganisms are killed on exposure to this range of hydrogen ion concentration.

Both mononuclear and polymorphonuclear phagocytic cells utilize glycolysis rather than respiration as their primary source of energy, even under aerobic conditions (35). Glycolysis results, of course, in production of lactic acid. Rabbit granulocytes produce large amounts of lactic acid, about 6 mg per gram wet weight of cells per hour. In all likelihood, the production and accumulation of lactic acid within leucocytes accounts, at least in part, for the low pH of their cytoplasm. As mentioned earlier in the present review, the combination of acid pH and lactic acid manifests antimicrobial effects *in vitro*. It seems reasonable to postulate that many gram-positive microbes are suppressed in growth or killed within phagocytes by lactate and hydrogen ions.

Phagocytic cells contain long chain fatty acids which are at least potentially antibacterial. A recent study on rabbit polymorphonuclear leucocytes revealed that the over-all nonesterified fatty acid content and composition of these cells is in general similar to that of other rabbit tissues (36). Granulocytes, therefore, do not appear to be endowed with unique antimicrobial fatty acids. However, since leucocyte cytoplasm is not known

to contain any albumin-like proteins, fatty acids therein might be free to exert toxic effects on microorganisms, in contrast to the situation in serum where they are "detoxified" by binding to albumin.

Granulocytes of certain animals contain very large amounts of veridoperoxidase. The porphyrin moiety of this molecule, if liberated from the protein by acid or otherwise, might well exert bactericidal effects on gram-positive bacteria in a manner similar to that of the heme porphyrins from blood pigment. No reliable data are available concerning this possibility.

Polymorphonuclear leucocytes contain at least three antimicrobial proteins: lysozyme, phagocytin, and histones.

Lysozyme is present in large amounts (1 to 2 mg per ml of packed cells) in granulocytes (37). It appears to be largely in the free state, since it can be extracted from disrupted rabbit cells with any of a variety of neutral or acid salt solutions. Furthermore, the lysozyme activity of these crude extracts suggests that lysozyme antagonists are not present in significant concentrations. The limited bacteriolytic spectrum of lysozyme raises some question as to its over-all importance as an antimicrobial factor of cells; however, as mentioned above, lysozyme acting in combination with other agents, such as chelators and acids, may exert noxious effects on a wide range of microorganisms. Whether or not such other agents are present in polymorphonuclear leucocytes in a form suitable for synergism with lysozyme is unknown.

Lysozyme, lactic acid, and low pH cannot explain all the antimicrobial action of granulocyte cytoplasm, because it exerts lethal effects on several gram-negative bacterial species not susceptible to these agents singly or in combination. These observations led to the discovery of another leucocytic antibacterial agent, almost certainly a protein, called phagocytin (38, 39). Recent studies not yet published show that only a small fraction, approximately 10 per cent, of the total phagocytin is extractable from disrupted polymorphonuclear leucocytes with neutral salt solutions. The remainder of this material passes into solution in weak acid (0.01 M citric acid) under conditions which leave nuclei largely intact. Phagocytin thus appears to be an acid-soluble cytoplasmic protein. It is present in large amounts in granulocytes of several species but is

not demonstrable in extracts of other tissues and cell types, including mononuclear phagocytes. Phagocytin kills a wide range of bacteria, both gram-negative and gram-positive, under laboratory conditions which imitate those within cells as well as present knowledge permits. Since polymorphonuclear white cells disrupted by freezing and thawing but otherwise untreated exhibit antibacterial activity similar to that of extracted phagocytin, it seems reasonable to assume that this agent may act *in vivo*.

Histones are also present in granulocytes, but according to present concepts of nucleoprotein solubility and stability, it is not likely that significant amounts of histone would be present outside the nucleus. Furthermore, the antibacterial activity of histone B is antagonized by conditions of ionic strength which approach those probably existing within cells, making it unlikely that histones play an important role as an antimicrobial factor in normal host cells.

There are many reports in the literature dealing with antibacterial activity of whole blood cell extracts, especially on gram-positive microorganisms. The name leukin, which has been used to signify the active material in these extracts, is avoided in the present review for the sake of precision and clarity. In reality, it seems likely that what has been called leukin represents the activity of various cell components, probably including phagocytin, histones, and related basic peptides (8, 40).

Considerable knowledge is, therefore, available concerning intracellular antimicrobial mechanisms of polymorphonuclear phagocytes. Low pH, lactic acid, long chain fatty acids, lysozyme, and phagocytin are all present and the chances seem good that several of these agents may act *in vivo*. Granulocytes may also contain other substances to deal with particular types of microorganisms not yet studied in this regard. Mononuclear phagocytes apparently possess no lysozyme or phagocytin, and nothing is known of their long-chain fatty-acid makeup. Low pH and lactic acid are the only antimicrobial factors at present known to exist in these cells. Probably much remains to be learned about mechanisms of bactericidal action in macrophage cytoplasm.

#### ANTIMICROBIAL FACTORS OF INFLAMED OR NECROTIC TISSUES

We have thus far dealt with antimicrobial action in normal tissues and phagocytic cells. In

reality, bacterial infection is almost always accompanied, even if only on a microscale, by inflammation or necrosis. Do new antimicrobial factors appear in this situation, or is the action of normal tissue agents changed in the inflammatory site? Very little definitive information is available on which to base an answer to these important questions.

No antimicrobial agent has yet been demonstrated to be exclusively associated with inflammation or necrosis. Stated in another way, there is no evidence for production in the inflammatory reaction of heretofore unrecognized substances toxic for microorganisms. However, it seems quite likely that inflamed or dead tissues provide a highly fertile area for liberation or action of some of the antimicrobial agents of normal tissues considered in a preceding section of this review. Lowered oxygen tension, increased carbon dioxide concentration, acidity, and glycolysis with formation of large amounts of lactic acid are all demonstrated characteristics of the inflammatory site, and, acting singly or more especially in combination, probably provide the tissues with increased capacity to combat microbial invaders after the battle has begun. Tissue breakdown, on a micro- or macroscale, may well lead to release of basic peptides, porphyrins, amines, fatty acids, and histones not available for antimicrobial action in healthy tissue. It thus is possible, even probable, that tissues are endowed with a reserve supply of antimicrobial substances which are called into action by the inflammation and necrosis resulting from the first engagement of the conflict.

Even on a cellular level, it is possible that maximum bactericidal activity of phagocytic cytoplasm does not exist preformed but rather develops in response to ingestion of foreign particles. This concept of "intracellular inflammation" would take into account such phenomena as altered phagocyte metabolism during and after engulfment, with the "burst of acidity" leading to a direct increase in antibacterial activity and secondarily increasing such activity by potentiation of the toxicity of lactic acid and perhaps lysozyme. Also, it may be recalled that in the normal granulocyte, phagocytin exists largely in bound form and can be liberated by exposure to dilute organic acid; the increased acid formed intracellularly following phagocytosis may well result in greater availability of phagocytin for antimicrobial action.

## CONCLUSION

Many antimicrobial substances have been found in tissues and cells of the mammalian host. Almost certainly other chemical agents which function to control multiplication of parasites *in vivo* remain to be discovered. There is much validity to the criticism that many of the antimicrobial substances from tissues and cells may act on bacteria only under laboratory conditions, and not in the living host. On the other hand, absolute proof that a chemical compound of natural origin exerts lethal effects on microorganisms *in vivo* is, in many instances, difficult or impossible to attain with present inadequate techniques and lack of information about the chemical micro-environment within cells and tissues. The time has probably now arrived when it would be wise for us to slacken the search for additional antimicrobial agents of tissues, and expend increased effort investigating interrelations between the many agents already known and the tissue environments in which they act. Synergists and antagonists for a given natural bactericidal substance are probably always present or readily available in an infected region. Our knowledge of the subtle and dynamic biochemical aspects of the local site of the host-parasite encounter is primitive indeed, yet it is likely that just these aspects determine the presence or absence of an antimicrobial effect.

With continuing observations in depth and breadth of the infectious process, it is becoming more and more evident that in many instances host resistance, not exposure to the parasite, is the prime determinant of disease. Many potentially pathogenic microbes, such as staphylococci and tubercle bacilli, are widely spread in the human population, yet produce disease only in the rare individual in whom the mechanisms of natural resistance have failed. Gross mechanisms of host resistance, such as phagocytes and antibodies, certainly deserve the important role assigned to them in the past. But neither of these essentially descriptive terms really provides us with knowledge needed to understand the true workings of defense mechanisms against microbes. In the final analysis the intimate biochemical environment, whether intracellular or extracellular, determines the fate of bacteria which reach tissue sites. The obvious technical difficulty of studying the complex biochemical environment in various areas of the host poses a difficult chal-

lenge *per se*. Furthermore, the factors in this environment of importance to antimicrobial action may differ for each microorganism or each group of microorganisms. The problems to be solved are formidable, but they must be solved before our concepts of pathogenesis of infection can be placed on a rational basis.

## REFERENCES

1. NEDZEL, A. J. AND ARNOLD, L. 1930 Influence of eggwhite upon the absorption of bacteria from the intestinal tract. *Proc. Soc. Exptl. Biol. Med.*, **28**, 358-360.
2. GORDON, L. E., RUMML, D., HAHNE, H. J., AND MILLER, C. P. 1955 Studies on susceptibility to infection following ionizing irradiation. *J. Exptl. Med.*, **102**, 413-424.
3. HAMMOND, C. W. AND MILLER, C. P. 1955 The incidence of endogenous bacteremia in X-irradiated rabbits. *Radiation Research*, **3**, 191-201.
4. ROUND, H., KIRKPATRICK, H. J. R., AND HAILS, C. G. 1936 Further investigations on bacteriological infections of the mouth. *Proc. Roy. Soc. Med.*, **29**, 1552-1556.
5. ELLIOTT, S. D. 1939 Bacteraemia and oral sepsis. *Proc. Roy. Soc. Med.*, **32**, 747-759.
6. MURRAY, M. AND MOOSNICK, F. 1941 Incidence of bacteremia in patients with dental disease. *J. Lab. Clin. Med.*, **26**, 801-802.
7. SCRIMSHAW, N. S., TAYLOR, C. E., AND GORDON, J. E. 1959 Interactions of nutrition and infection. *Am. J. Med. Sci.*, **237**, 367-403.
8. SKARNES, R. C. AND WATSON, D. W. 1957 Antimicrobial factors of normal tissues and fluids. *Bacteriol. Revs.*, **21**, 273-294.
9. CORPER, H. J., LURIE, M. B., AND UYEI, N. 1927 The variability of localization of tuberculosis in the organs of different animals. *Am. Rev. Tuberc.*, **15**, 65-87.
10. DUBOS, R. J. 1953 Effect of the composition of the gaseous and aqueous environments on the survival of tubercle bacilli *in vitro*. *J. Exptl. Med.*, **97**, 357-366.
11. VALLEY, G. 1928 The effect of carbon dioxide on bacteria. *Quart. Rev. Biol.*, **3**, 209-224.
12. DAVIES, R. 1940 The effect of carbon dioxide on the growth of the tubercle bacillus. *Brit. J. Exptl. Pathol.*, **21**, 243-253.
13. NIEMAN, C. 1954 Influence of trace amounts of fatty acids on the growth of microorganisms. *Bacteriol. Revs.*, **18**, 147-163.
14. DUBOS, R. J. 1950 The effect of organic acids on mammalian tubercle bacilli. *J. Exptl. Med.*, **92**, 41-54.

15. DUBOS, R. J. 1953 Effect of ketone bodies and other metabolites on the survival and multiplication of staphylococci and tubercle bacilli. *J. Exptl. Med.*, **98**, 145-155.
16. DUBOS, R. J. 1954 *Biochemical determinants of microbial diseases*. Harvard University Press, Cambridge, Mass.
17. FLEMING, A. 1922 On a remarkable bacteriolytic element found in tissues and secretions. *Proc. Roy. Soc. (London)*, **93**, 306-317.
18. SALTON, M. R. J. 1957 The properties of lysozyme and its action on microorganisms. *Bacteriol. Revs.*, **21**, 82-99.
19. REPASKE, R. 1956 Lysis of gram negative bacteria by lysozyme. *Biochim. et Biophys. Acta*, **22**, 189-191.
20. BLOOM, W. L., WATSON, D. W., CROMARTIE, W. J., AND FREED, M. 1947 Preparation and characterization of an anthracidal substance from various animal tissues. *J. Infectious Diseases*, **80**, 41-52.
21. SKARNES, R. C. AND WATSON, D. W. 1956 Characterization of an antibacterial peptide from calf thymus. *Proc. Soc. Exptl. Biol. Med.*, **93**, 267-269.
22. HIRSCH, J. G. AND DUBOS, R. J. 1954 Chemical studies on a basic peptide preparation derived from calf thymus. *J. Exptl. Med.*, **99**, 65-78.
23. BLOOM, W. L., WINTERS, M. G., AND WATSON, D. W. 1951 The inhibition of two antibacterial basic proteins by nucleic acids. *J. Bacteriol.*, **62**, 7-13.
24. HIRSCH, J. G. 1954 Mechanisms involved in the antimycobacterial activity of certain basic peptides. *J. Exptl. Med.*, **99**, 79-88.
25. CRAMPTON, C. F., MOORE, S., AND STEIN, W. H. 1955 Chromatographic fractionation of calf thymus histone. *J. Biol. Chem.*, **215**, 787-801.
26. HIRSCH, J. G. 1958 Bactericidal action of histone. *J. Exptl. Med.*, **108**, 925-944.
27. HOBSON, D. AND HIRSCH, J. G. 1958 The antibacterial activity of hemoglobin. *J. Exptl. Med.*, **107**, 167-183.
28. HIRSCH, J. G. AND DUBOS, R. J. 1952 The effect of spermine on tubercle bacilli. *J. Exptl. Med.*, **95**, 191-208.
29. ROZANSKY, R., BACHRACH, U., AND GROSSOWICZ, N. 1954 Studies on the antibacterial action of spermine. *J. Gen. Microbiol.*, **10**, 11-16.
30. HIRSCH, J. G. 1953 The essential participation of an enzyme in the inhibition of growth of tubercle bacilli by spermine. *J. Exptl. Med.*, **97**, 327-344.
31. CARVAJAL, G. AND CARVAJAL, E. J. 1957 *Beta-propylal gamma-butylal-imine*; new substance having inhibitory effect on *Mycobacterium tuberculosis* var. *hominis* H37Rv. *Am. Rev. Tuberc. Pulmonary Diseases*, **76**, 1094-1096.
32. GROSSOWICZ, N., RAZIN, S., AND ROZANSKY, R. 1955 Factors influencing the antibacterial action of spermine and spermidine on *Staphylococcus aureus*. *J. Gen. Microbiol.*, **13**, 436-441.
33. SOLTYS, M. A. 1953 An antituberculous substance in tuberculous organs. *J. Comp. Pathol.*, **63**, 147-152.
34. METCHNIKOFF, E. 1893 *Lectures on the comparative pathology of inflammation*. Translated from the French by F. A. Starling and E. H. Starling. Kegan Paul, Trench, Trüber and Co., London.
35. SUTER, E. 1956 Interaction between phagocytes and pathogenic microorganisms. *Bacteriol. Revs.*, **20**, 94-132.
36. ELSBACH, P. 1960. Composition and synthesis of lipids in resting and phagocytized leukocytes, *J. Exptl. Med.*, **110**, 969.
37. MYRVIK, Q. N. AND WEISER, R. S. 1955 A serum bactericidin for *Bacillus subtilis*. *J. Immunol.*, **74**, 9-16.
38. HIRSCH, J. G. 1956 Phagocytin: a bactericidal substance from polymorphonuclear leukocytes. *J. Exptl. Med.*, **103**, 589-611.
39. HIRSCH, J. G. 1956 Studies on the bactericidal action of phagocytin. *J. Exptl. Med.*, **103**, 613-621.
40. SKARNES, R. C. AND WATSON, D. W. 1956 Characterization of leukin: an antibacterial factor from leukocytes active against gram positive pathogens. *J. Exptl. Med.*, **104**, 829-845.

## DISCUSSION

Previous work on the lysozyme content of peritoneal macrophages indicated that the lysozyme content of these cells was low or absent. Alveolar macrophages, however, procured by washing the trachea of normal rabbit lung with balanced salt solution, contain large amounts of lysozyme: 2000 to 4000  $\mu\text{g}$  per ml of packed alveolar cells. These macrophages do not resemble peritoneal macrophages and to some degree resemble plasma cells. However, they are phagocytic, and because of their high lysozyme content must be considered to be a different type of macrophages than those derived from the peritoneal cavity. There must, therefore, be families of macrophages that have

different enzymatic or antibacterial components and perhaps varying abilities to kill microorganisms (Myrvik, Charlottesville).

Recently, Jolle (reference 18) has crystallized lysozyme from dog spleen and shown it to have twice the enzymatic activity of egg-white lysozyme. However, it is not known if other mammalian lysozymes have similarly greater enzymatic action. On the other hand, differences have been demonstrated between the chemical characteristics and spectrum of activity of lysozyme from plants and egg-white (Thompson, R., 1940, Lysozyme and its relation to antibacterial properties of various tissues and secretions, Arch. Pathol., **30**, 1096-1134). Furthermore, leucocytic and egg-white lysozymes may differ from each other (Dubos and Hirsch, *unpublished observa-*

*tions*). It seems likely, therefore, that lysozymes from various sources, although similar, nevertheless have distinguishing characteristics.

Cellular and tissue factors in resistance may influence the progress of inflammation but not the establishment of infection. In addition, different types of infection, different types of microorganisms, and the physiological state of the host and bacterium will undoubtedly influence the character of the lesion. The effects of these factors upon the progress of the infection is clearly illustrated in tuberculosis, where under most circumstances in man this microorganism does not produce progressive disease. Changing the local conditions, however, can result in a new set of influences that can change the outcome of the infection (Dubos, New York).